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VIEWPOINT

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BAG3: An enticing therapeutic target for idiopathic pulmonary fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a dreadful and fatal disease of unknown etiology, for which no cure exists. Autophagy, a lysosomal cellular surveillance pathway is insufficiently activated in both alveolar epithelial type II cells and fibroblasts of IPF patient lungs. Fine-tuning this pathway may result in the degradation of the accumulated cargo and influence cell fate. Based on our previous data, we here present our view on modulating autophagy via a unique co-chaperone, namely Bcl2-associated athanogene3 (BAG3) in IPF and discuss about how repurposing drugs that modulate this pathway may emerge as a promising novel therapeutic approach for IPF.

KEYWORDS

autophagy, BAG3, idiopathic pulmonary fibrosis, therapeutic intervention

1 | IDIOPATHIC PULMONARY FIBROSIS AND AUTOPHAGY

Idiopathic pulmonary fibrosis (IPF) is an aggressive form of organ fibrosis with unknown etiology.[1] Patients display progressive decline in lung function and only a few years of survival rate after diagnosis.[1,2] Histologically, IPF lung shows the pattern of usual interstitial pneumonia with hyperplastic alveolar epithelial cells type II cells (AT2) underlying the hyperproliferative fibroblasts. Mounting evidence suggests that repeated AT2 injury and aberrant alveolar–mesenchymal crosstalk leads to collagen deposition and damage to the delicate alveolar architecture, ultimately resulting in progressive dyspnea, lung function decline, and death.[2] IPF shows a remarkable age-related onset and hence the pathological significance of several aging-associated signaling mechanisms is reported in several cell types of IPF lungs. In that, the lysosome-dependent cellular surveillance pathway termed autophagy has been shown to be defective in several IPF cells, including AT2, fibroblasts and alveolar macrophages.[3–5] Autophagy pathway is majorly responsible for the turnover of long-lived proteins and damaged organelles to generate energy and raw material for

regenerating cellular structures to fight against any physiological alterations or injury, thereby improving cellular and organismal life-span. A full description of autophagy is out of the scope of this article and the reader is directed to some excellent reviews.[6,7] Poly-ubiquitinated proteins, enhanced sequestosome1 (SQSTM1/p62) with insufficient autophagy and mitophagy are reported in fibroblasts as well as AT2 of IPF lungs, in both spontaneous[3,4] and familial forms and in animal models of lung fibrosis[8–11] (Figure 1). Approved IPF therapy drugs, Pirfenidone (Prif) and Nintedanib (Nin), both trigger autophagy and/or mitophagy.[12,13] All in all, a protective role for autophagy has been ascertained in the context of lung fibrosis. Inhibition of mTOR using rapamycin did show anti-fibrotic effects in a TGF-β dependent manner.[14,15] From the autophagy perspective, mice with loss of *Unc-51 like autophagy activating kinase* (*Ulk1*/2) or *Atg5* display lung defects at an early age [16] and *Atg4−/−* and *microtubule-associated proteins 1A/1B light chain 3B−/−* (*LC3B−/−*) mice display susceptibility to lung fibrosis.[17,18] While rapamycin treatment has exerted beneficial effects to some extent in the context of lung fibrosis, such direct targeting of autophagy may prove vulnerable to cells, and the already stressful environment in the IPF lungs deserve a more modulating/fine-tuning effect. In this regard, we aimed at modulating autophagy via its regulators. Our analysis revealed one well-studied

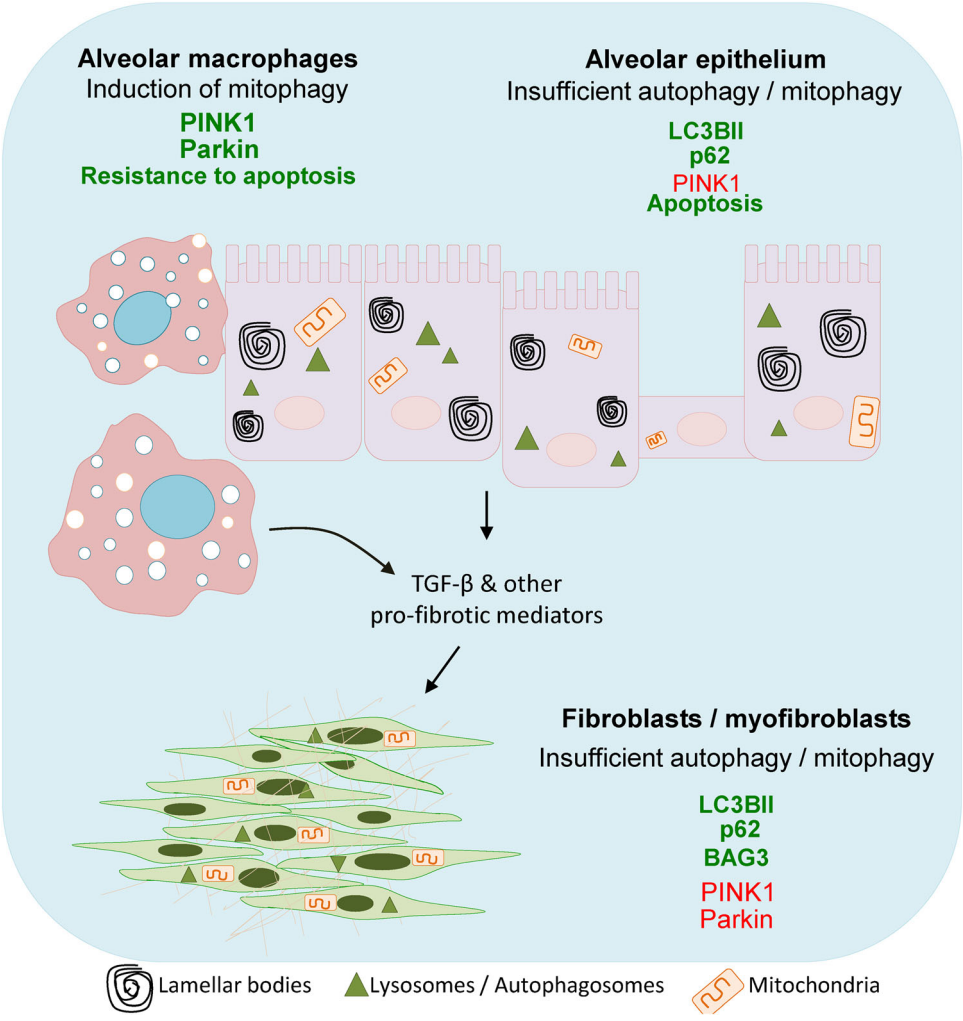

FIGURE 1 Schematic depicting the current knowledge of autophagy in different cells of idiopathic pulmonary fibrosis (IPF) lungs. Shown here are alveolar epithelial cells (AT1 and AT2 cells), macrophages and fibroblasts. Both AT2 cells and fibroblasts display insufficient autophagy (macroautophagy) with an increase in LC3BII and p62 proteins and defective mitophagy due to the loss of PINK1 and/or Parkin proteins. Macrophages, on the other hand, show increased mitophagy via PINK1 or Parkin and display resistance to apoptosis. The dying AT2 cells and autophagy-active macrophages secrete TGF-β and many pro-fibrotic factors which facilitate the exaggerated proliferation of fibroblasts in the IPF lung. Green font is used to depict an increase and red font is used to depict a decrease of the indicated proteins. For ease of understanding, other cellular stress mechanisms that are prevalent in IPF cells are not shown.

autophagy-regulating protein and a unique co-chaperone, the Bcl2-associated athanogene-3 (BAG3).

2 | BAG3-MEDIATED AUTOPHAGY AND ITS MODULATION IN IPF

BAG3 is one of the six members of the BAG superfamily and distinguishes itself from the other members by the presence of a WW domain at its N-terminus that is required for the induction of BAG3-dependent autophagy. BAG3 participates in a myriad of signaling events by stimulating its chaperone, Hsc70, and regulating its client binding activity. In a complex with Hsc70 and Hspb8, it delivers misfolded ubiquitinated proteins to the phagophore and aids its maturation into the autophagosome. BAG3 binds to the HSPA8-associated ubiquitin ligase STUB1/CHIP and its partner ubiquitin-conjugating enzyme E2D (UBE2D) and helps to ubiquitinate chaperone-bound filamin C (FLNC), an isoform of filamin protein, thereby leading to its degradation by autophagy by recruiting p62 in striated skeletal muscles.[19–21] It is because of its involvement in a plethora of cellular signaling pathways, the pathomechanistic role of BAG3 has been reported in many aging-associated diseases, and mutations in *BAG3* gene were shown to destabilize Hsc70, resulting in cardiomyopathy. Likewise, we recently reported elevated BAG3 protein, but a defect in BAG3-dependent autophagy in IPF fibroblasts versus those of healthy lungs.[22] Drugs modulating BAG3, namely 5-azacytidine (5-Aza) and Cantharidin (Ctd), in combination with the IPF therapy drug Pirfenidone (Pirf) mitigated fibroblast proliferation as well as collagen deposition in IPF-derived precision-cut lung slices (PCLS). While Pirf is an established IPF therapy drug with autophagy-inducing effects, 5-aza is a demethylating agent and has been shown to inhibit IPF fibroblast proliferation. Its effect on other cell types remains elusive. Ctd, a terpenoind has been reported to inhibit cancer cell growth by influencing BAG3 and its chaperones. In IPF fibroblasts, we observed a decrease in BAG3 protein upon Ctd treatment.[22] We further asked if BAG3 protein is altered in the AT2 of IPF patients or if the increase is restricted to fibroblasts of IPF patient lungs. Interestingly, immunofluorescence for BAG3 protein on paraffin-embedded IPF as well as healthy donor lung sections revealed a significantly elevated staining for BAG3 in the IPF AT2 (Figure 2). Of note, macroautophagy has been shown to be defective in both IPF fibroblasts & AT2. The defective BAG3-dependent autophagy in IPF fibroblasts or the increase in BAG3 protein in IPF AT2, as observed in our study, may be either due to (a) an inefficient recruitment of p62 onto the phagophores or (b) the inability of these cells to fully perform the final steps of autophagy, in either case resulting in the accumulation of the BAG3-dependent autophagic cargo in these cells. How the increase in BAG3 protein leads to the differential regulation of cell fate of these two cell types remains to be answered.

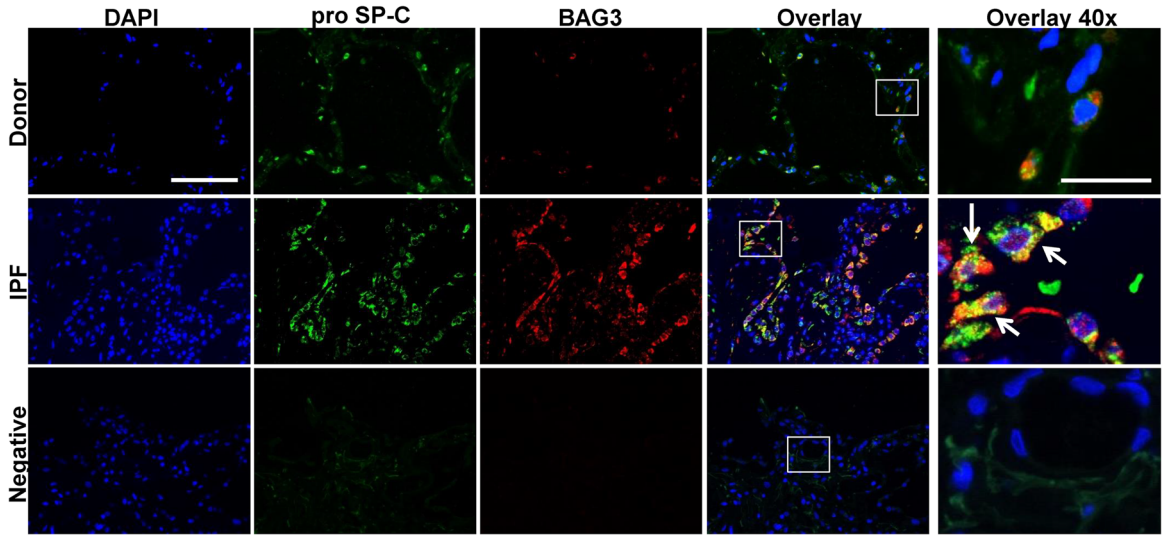

FIGURE 2 BAG3 protein is increased in AT2 cells of idiopathic pulmonary fibrosis (IPF) lungs. Paraffin lung sections from IPF and age-matched donor lungs were stained for the AT2 marker, pro-SP-C (green), and for BAG3 (red). Nuclear staining (blue) was performed using 4′,6-diamidino-2-phenylindole (DAPI). Scale bar = 100 μm. Far right panel indicates high-magnification images (40x). Arrows indicate pro-SP-C positive AT2 cells stained for BAG3 in IPF lungs. Scale bar = 25 μm. Sections where primary antibodies were omitted served as negative controls.

3 | BAG3-MEDIATED AUTOPHAGY: A FUTURE THERAPEUTIC TARGET IN IPF?

It has been elegantly shown that Ctd inhibits the activity of heat shock factor 1 (HSF1). Since *BAG3* is an HSF1-inducible gene, the heat-shock induced, but not the basal *BAG3* mRNA was downregulated by Ctd treatment,[17] indicating that Ctd might exert its effects differently in cells stressed with heat-shock. Likewise, the heat-shock-induced BAG3 protein levels were also decreased upon Ctd treatment in a dose-dependent manner. In agreement, our study[22] also showed that Ctd treatment did not alter the mRNA levels of *BAG3* but did decrease its protein levels significantly besides an increase in autophagy flux and decreased proliferation of IPF fibroblasts (no heat-shock treatments). Further, in IPF fibroblasts that were knocked down for *BAG3* gene, Ctd treatment did not show similar effects, indicating that Ctd exerts its beneficial effects at least in part via BAG3 protein in IPF fibroblasts. In addition, Ctd promoted a downregulation of FLNC, a substrate of BAG3-mediated autophagy. After a very careful and thorough review of previous reports and our own observations, one lesson that we derived is that: inhibiting BAG3 gene by knocking it down is not beneficial, but modulating BAG3-mediated autophagy via drugs like Ctd is beneficial for IPF fibroblasts.

On a cautionary note, BAG3 modulators like Ctd are toxic to administer systemically.[18] Hence proof-of-concept experiments may include this drug, but it may not be a choice for pulmonologists to include it in its current form into clinical trials. Usage of very low dose Ctd alongside with the IPF standard of care therapies or

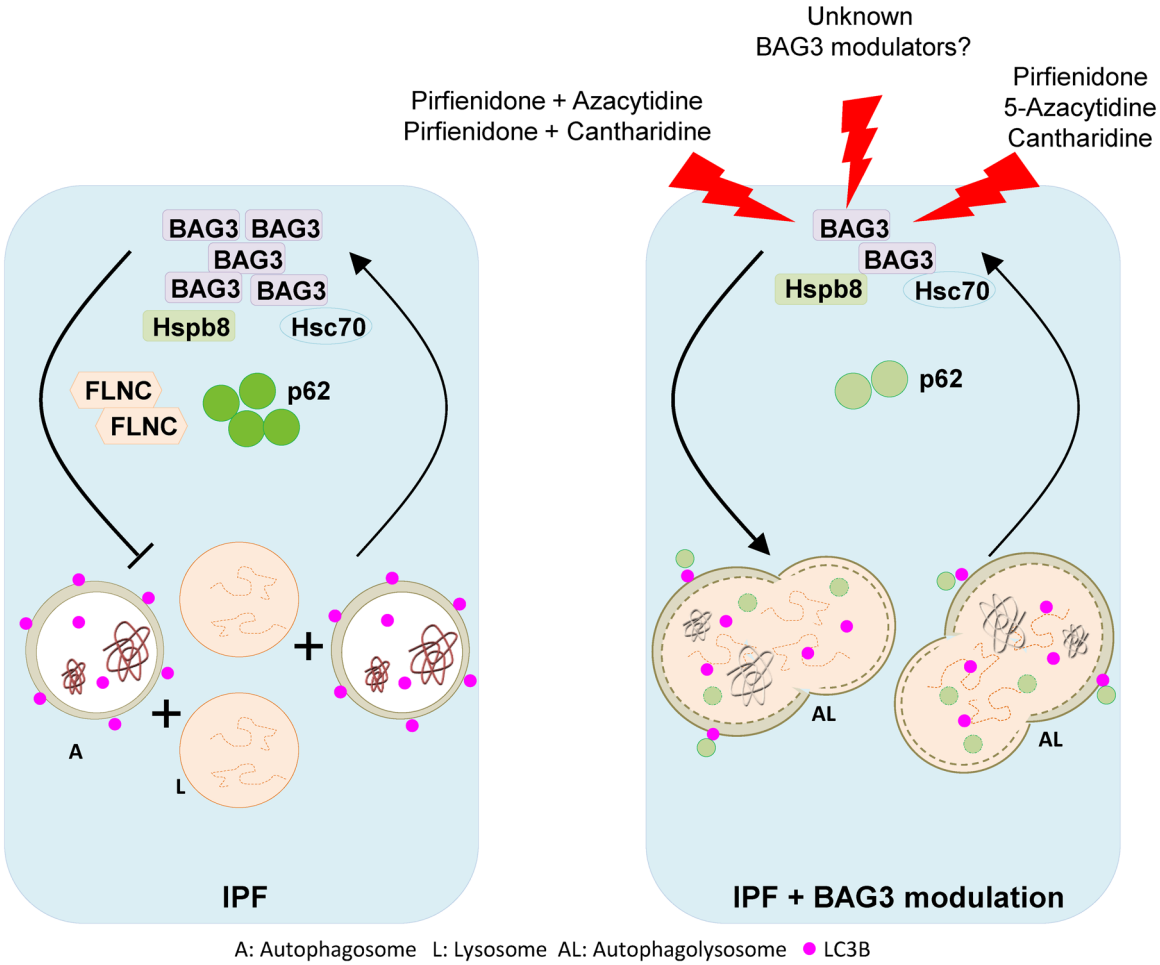

FIGURE 3 Partly hypothetic view of BAG3 modulation in idiopathic pulmonary fibrosis (IPF): On the left, situation in an IPF cell (either AT2 or fibroblast) is shown where BAG3, FLNC as well as p62 are increased. p62 is not recruited to autophagosomes, and aggregates are not degraded. On the right side, an IPF cell upon BAG3 modulation is shown where treatment with the indicated BAG3 modulating drugs results in the recruitment of p62 by BAG3 into the autophagy pathway, and since these drugs induce autophagic flux, subsequent fusion of autophagosomes and lysosomes occur, thereby leading to the degradation of the cargo. HSPB8 and Hsc70 are also depicted that are suggested to form a complex with BAG3 during BAG3-mediated autophagy.

development of safer derivatives of Ctd [23] in the near future may overcome this problem. Likewise, 5-aza which also modifies BAG3-mediated autophagy, is a clinically well-tolerated drug and is a first-choice of therapy for myelodysplasia (MDS). However, pneumonitis, interstitial lung disease, and acute lung injury are reported in some studies but not in clinical trials. On the contrary, 5-aza treatment has been shown to reduce IPF fibroblast proliferation,[24] a phenomenon observed in our study as well.[22] After a careful review, we believe that the already known drugs that modulate BAG3 with less toxic effects may serve the purpose (Figure 3). A hunt for additional small molecules to target BAG3 might prove beneficial.

4 | PERSPECTIVE

Given that manipulation of BAG3-mediated autophagy has already been considered for several diseases, namely cancers, myopathies, neurodegenerative diseases, and cystic fibrosis lung disease, BAG3 remains an enticing target for IPF therapy. In addition, following a repurposing concept, such drugs could be easily considered for clinical exploitation. We believe that the translational relevance of drugs modulating BAG3-mediated autophagy in IPF may advance our understanding to extend these targets to other chronic lung diseases.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT
The study protocol was approved by the Ethics Committee of the Justus-Liebig-University School of Medicine (111/08 and 58/15). All patient-related biomaterials were provided by the UGMLC Giessen Biobank and the European IPF Registry/Biobank.

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